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VESICANT STUDIES
II. VOLATILITY DETERMINATIONS OF BUTYL 2-CHLOROETHYL SULFIDE ON VARIOUS SURFACES, INCLUDING PIG SKIN

JUNE JAEGER, BS
and
PETER SCHMID, Ph.D.

DIVISION OF CUTANEOUS HAZARDS

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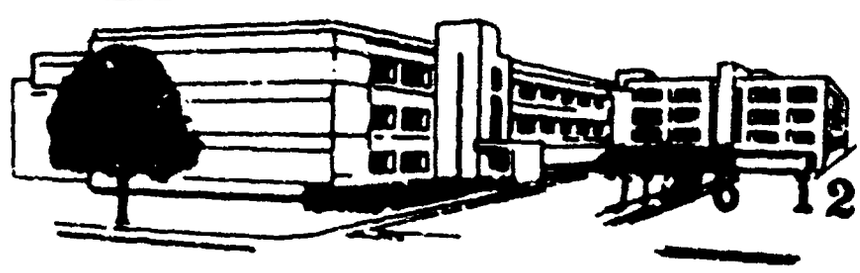

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Vesicant Studies. II. Volatility Determinations of Butyl 2-Chlorethyl Sulfide on Various Surfaces, Including Pig Skin--Jaeger et al.

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Volatility studies on butyl 2-chloroethyl sulfide indicate that this compound would be a suitable analog for vesicant studies in place of mustard gas, which is a surety agent. Its vapor pressure was found to be 0.099 mm Hg. at 20°C. It is not retained on filter paper; it appears to be covalently bound to silica gel, and it volatilizes from full thickness pig skin surface at a rate of 50% relative to the same amount of butyl 2-chloroethyl sulfide leaving filter paper under the same conditions during the same period of time.

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Vesicant Studies. II. Volatility Determinations of Butyl 2-Chloroethyl Sulfide on Various Surfaces, Including Pig Skin--Jaeger

INTRODUCTION

Before research studies on the effects of vesicants on skin could be started, it was necessary to find a substitute for bis-2-chloroethyl sulfide (mustard gas), which is a surety agent, to simulate its biological and physical properties. A perusal of the literature led us to select butyl 2-chloroethyl sulfide, a known vesicant, (CAS Reg. 4303-40-6) (BCS) as a candidate compound (1-6).

Carbon-14 labeled BCS was custom-synthesized by ICN, Irvine, CA, with a specific activity of 10.97 mCi/mmole. Both carbons of the chloroethyl group are labeled. A gas-liquid chromotography chromatogram of this compound showed one peak with a retention time coinciding with a "standard" cold sample injected just before the labeled compound was injected. Attempts to determine its purity by thin-layer chromatography on Whatman LHK-P plates, after it had been stored in the refrigerator for one year, resulted in an unexplained loss of about 60% of the radioactivity deposited at the origin. After the plates were developed, the spots were located and scraped into scintillation counting fluid, and the radioactivity was measured. A possible explanation for this loss was the relatively high volatility of BCS. To test this hypothesis, a known amount of ^{14}C-BCS was applied to two types of silica gel plates and after a predetermined interval of time, it was scraped off and placed in a liquid scintillation solution for radioisotope measurements. A preliminary attempt at measuring the vapor pressure of ^{14}C-BCS indicated a value similar to the bifunctional mustard, bis-2-chloroethyl sulfide (7).

The following report describes the results of our experiments dealing with loss of ^{14}C-BCS from various surfaces and an estimation of the vapor pressure of BCS.

METHODS

Kinetics of the loss of ^{14}C-BCS from an LHK-P Thin-Layer Plate (Phase I)

Four microliters of ^{14}C-BCS (1.20 μCi/μl) were delivered on a prescribed 64 mm^2 area of an LHK-P (Whatman) thin-layer plate in each of four separate rows by a Hamilton 10-μl syringe. The plates then remained exposed to ambient air in the hood at 20°C for 30 minutes.

After 30 minutes, the material in the delineated areas was scraped into screw-cap vials containing 10 ml of Opti Fluor® counting fluid. A blank containing silica gel from an area of 64 mm^2 and a control of 4 µl of ^{14}C-BCS delivered directly into 10 ml of Opti Fluor® in a scintillation vial to avoid any evaporation were also prepared. All samples were counted in a packard Tri Carb Model 4530. Identical 4 µl aliquots of ^{14}C-BCS and blanks were done on other LHK-P thin-layer plates and exposed to ambient air at 20°C in a hood for 1 to 3 minutes, and 17 hours. At no time was the fan turned on in the hood while volatility measurements were being made.

Table 1 shows that 35% of the recoverable activity is lost after 1 to 3 minutes relative to the control. Recovery of the radiolabel declines only slightly over the course of the next 17 hours; less than 50% of the applied radioactivity can be recovered after 17 hours.

Comparison of Loss of Radioactivity of ^{14}C-BCS from Various Surfaces during a 15-minute Interval (Phase II)

A second phase of this study was done to determine if the volatility varies with different substrates and/or surfaces. The same procedure as in Phase I was followed by using Whatman reverse-phase TLC KC18F plates and Whatman No. 1 filter paper strips. The strip was suspended in air, both during spotting of the sample and the timed interval of exposure to ambient air in the hood. The delineated 64 mm^2 area of filter paper was cut out with a pair of scissors, the square dropped into 10 ml of Opti Fluor® and the dpm were recorded. For this phase, all surfaces containing aliquots of ^{14}C-BCS were exposed to ambient air for 15 minutes.

The results are given in Table 2. Ninety-three percent of radioactivity is lost from filter paper; whereas, loss from the two silica gels is considerably less. These results are consistent with the suggestion that BCS forms covalent bonds with these dry silica gel surfaces, but not with Whatman No. 1 filter paper. It is also possible that BCS is held by adsorption, in which case a study of the temperature effect may lead to a plausible explanation of this phenomenon.

Measurement of Loss of Radioactivity from Various Surfaces by Surface Counting (Phase III)

During the third phase of this study, the rate of surface evaporation of ^{14}C-BCS was measured by using a portable isotope counter (Berline ESP 1 with an HP probe having a diameter of 70 mm and a height of 22.7 mm). A measured aliquot of ^{14}C-BCS was applied exactly as previously described on the following four surfaces: Whatman No. 1 filter paper, KC18F thin-layer plates, silica gel G thin-layer plates,

and a small piece of fresh full-thickness weanling female Yorkshire pig skin (shaved). The samples were placed sequentially on the stage of a laboratory jack, as shown in Fig. 1; the lower surface of the detector was approximately 5 mm from the surface of the material to be counted. Two microliters of neat ^{14}C-BCS (1.20 µCi/µl) were applied to the center of the area previously marked by a pen, or pinpoints for silica gel. At various frequent intervals, the radioactivity (dpm) was measured; the average dpm was calculated and recorded. After five measurements, the material containing the isotope was removed from the stage so the surface would be exposed to the air flowing through the hood and, simultaneously, the counting surface of the detector was cleaned by sweeping a fine stream of compressed air over it. This prevented build-up of background counts between the timed intervals of measuring radioactivity (Fig. 1).

From the graph produced in Figure 2, it can be concluded that the rate of loss of radioactivity per unit of time of ^{14}C-BCS from different surfaces is varied. Table 3 shows the percentage loss of the isotope from various surfaces relative to Whatman No. 1 filter paper after 15 minutes, which was arbitrarily set at 100%; the data are derived from Figure 2. The loss from pig skin is substantially greater for a given period of time than from the silica gels. These observations may explain why minerals such as fuller's earth are suitable as decontaminants as long as the vesicant has not penetrated into the skin, but is still loosely held at the surface.

Preliminary Values for the Vapor Pressure of BCS (Phase IV)

In the fourth phase of this study, a preliminary analysis of the static headspace concentration of ^{14}C-BCS was made (7). In this indirect method for determining volatile constituents in a liquid in a closed system, replicate samples of 20 µl of neat ^{14}C-BCS (0.012 µCi/µl) were injected with a 50-µl Hamilton syringe into 12.5-ml screw-cap flasks fitted with Mininert® valves in the caps and allowed to stand at 20°C. After one hour, 200 µl of the vapor phase was removed from each flask with a 500-µl Hamilton gas-tight syringe and injected into a scintillation vial containing 10 ml Opti Fluor® fluid each. Two controls were prepared by delivering 4 µl of ^{14}C-BCS (0.012 µCi/µl) directly into 10 ml Opti Fluor® in a scintillation counting vial. Measurement of the radioactivity in these samples was done the same way as described in Phase I and II of this report, and the following calculations were made:

20 µl neat ^{14}C-BCS = 0.012 µCi/µl
Specific activity = 0.1813 µCi/µmole

(a) Counts in the gas phase:

200 µl gas phase #1 = 616 dpm
#2 = 168 dpm
#3 = 364 dpm
mean = 383 dpm

Mean total activity in 12,500 µl gas phase = 23,938 dpm

(b) Control counts:

4 µl ^{14}C-BCS #5 = 92,465 dpm
#6 = 93,495 dpm
mean = 92,980 dpm

corresponding to 464,900 dpm for 20 µl of solution

(c) Vapor concentration:

$$c = \frac{(\text{wt. } ^{14}\text{C-BCS}) \times \text{dpm gas phase}}{\text{dpm control} \times \text{volume}} = \frac{\text{mass}}{\text{vol}}$$

$$c = \frac{202.0\ \mu g \times 23{,}938\ \text{dpm}}{464{,}900\ \text{dpm} \times 12.5\ \text{ml}} = 0.8321\ \mu g\ \text{BCS/ml of vapor}$$

(d) Vapor pressure:

$$p = \frac{nRT}{V}$$

M.W. = 152.67

$$R = 82.05\ \frac{\text{ml atm}}{^{\circ}\text{K mole}}$$

V = 12.5

T = 293°K

$n = 6.794 \times 10^{-4}$ moles

$$p = \frac{6.794 \times 10^{-4}\ (82.05)\ (293)}{12.5}$$

$p = 1.307 \times 10^{-4}$ atm = 0.099 mm Hg.

Our determinations indicate that at 20°C the vapor concentration in the vessel is about 0.83 µg/ml, which corresponds to a partial pressure of about 0.1 mm Hg. The vapor concentration of bis-2-chloroethyl sulfide is 0.625 µg/ml at 20°C (9).

CONCLUSIONS

It can be concluded that butyl 2-chloroethyl sulfide is not only a vesicant (9), but also has a molecular weight and vapor pressure (10) comparable to that of bis-2-chloroethyl sulfide. In our vesicant studies program, and based on these physical properties, we determined that butyl 2-chloroethyl sulfide was a suitable analog for mustard gas.

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LEGENDS

Fig. 1. Drawing representing the Eberline instrument and apparatus used to measure the dpm of ^{14}C-BCS on various surfaces.

Fig. 2. Graph of dpm of ^{14}C-BCS on (▲) Whatman No. 1 filter paper, (□) full thickness female weanling Yorkshire pig skin, (○) Whatman LHK-P silica gel, (●) Whatman reverse-phase KC18F silica gel from 0 to 132 minutes.

Figure 1

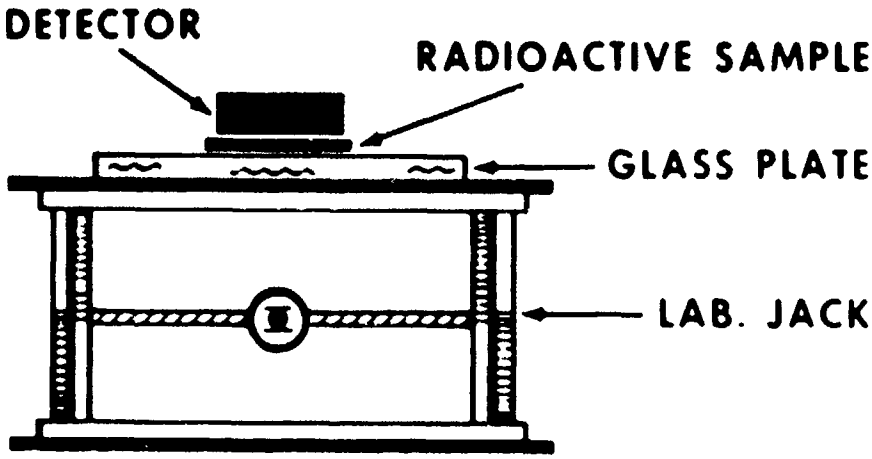

Figure 2

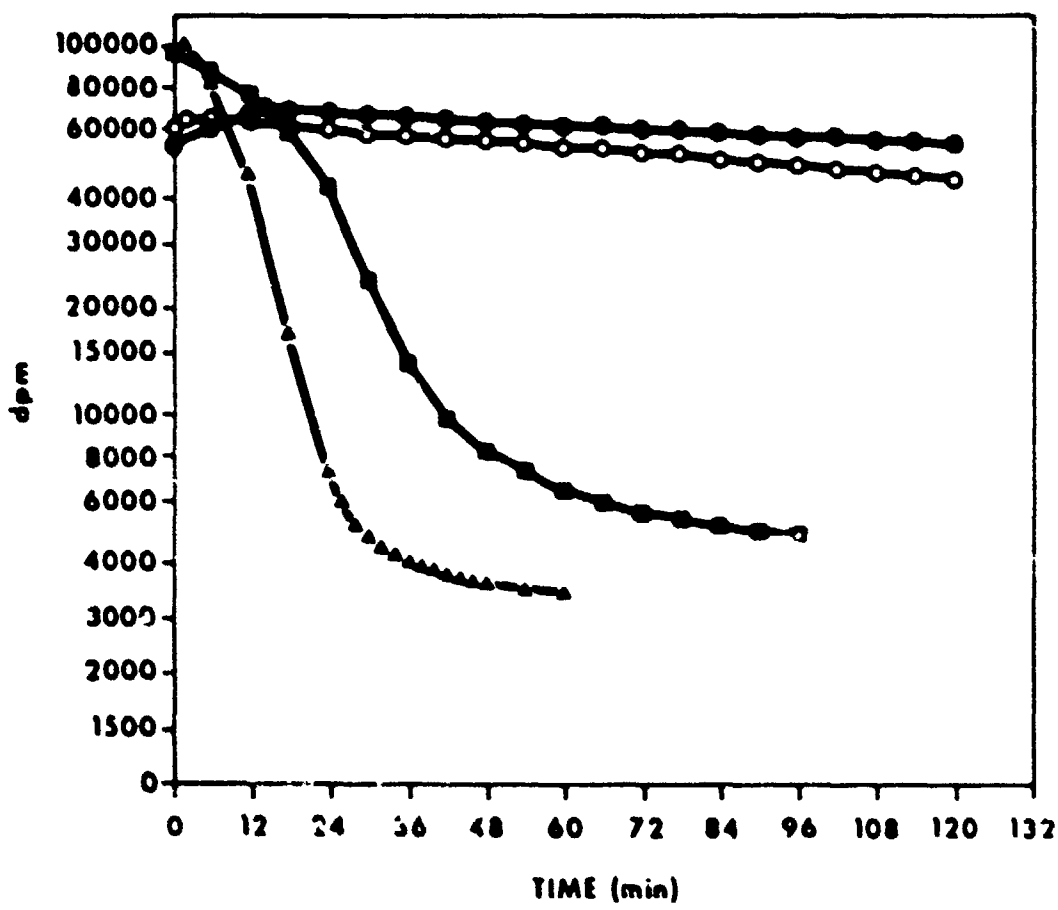

Table 1

Volatility of ^{14}C-BCS on LHK-P Silica Gel Layers (250 microns)

Time of Exposure	Number of Determinations	Mean % of BCS Remaining	Coefficient of Variation (C.V.)
Control 0 min.		100	
1 - 3 min.	4	65.0	9.1%
15 - 18 min.	3	67.4	3.3
30 - 33 min	4	56.3	2.7
17 hrs.	4	46.8	3.2

Table 2

Comparison of Volatility of ^{14}C-BCS on Various Surfaces During a 15 Minute Exposure to Ambient Air

Surface	No. of Determinations	Percent ^{14}C-BCS Remaining	Coefficient of Variation
RP KC18F silica gel	3	60.4	1.3%
Whatman No. 1 filter paper	3	7.1	11.3
LHK-P silica gel	3	67.4	2.7

Table 3

Relative Rate of Loss of Radioactivity on Various Surfaces as Measured with an Eberline ESP 1 Detector (Data Derived from Fig. 2)

Surface	Time Interval	Relative Rate of Loss of ^{14}C-BCS
Whatman No. 1 filter paper	6 - [illegible] min.	100.
Pig skin	20 - 56 min.	53.
Pig skin	2 - 20 min.	30.
LHK-P silica gel	2 - 120 min.	2.5
RP KC18F silica gel	14 - 120 min.	1.6